Bull Environ Contam Toxicol (2007) 79:95–98
DOI 10.1007/s00128-007-9140-8

A Study to Evaluate Heavy Metals and Organochlorine Pesticide Residue in *Zingiber officinale* Rosc. Collected from Different Ecological Zones of India

Chetna Mishra · Sapna Sharma · Poonam Kakkar

Received: 3 January 2007 / Accepted: 16 March 2007 / Published online: 11 May 2007

Products derived from medicinal plants have been used for therapeutic purposes for centuries. Herbal products are generally considered safe and have been proven effective against certain ailments. They are also extensively used, particularly in many Asian, African and other developing countries (Patwardhan et al., 2005). There is an urgent need to establish the identity, purity and quality of herbal products in order to have full efficacy and safety. Plant materials are liable to contain pesticide residues that accumulate due to different agricultural practices, soil treatment and storage practices, such as administration of fumigants. Widespread presence of heavy metals in soil, due to geoclimatic conditions and environmental pollution is inevitable, therefore their bioaccumulation in plants occurs. Organochlorine pesticides such as isoforms of hexachlorocyclohexane (HCH), metabolites of DDT, α-endosulphan and heavy metals, including non-volatile and volatile metals, have the potential to cause toxicity to liver and kidney, and may impair oxygen transport in blood. Our laboratory has been involved in the estimation of contaminants in medicinal plants. Levels of heavy metal/trace elements have been reported in some therapeutically important Indian medicinal plants (Rai et al., 2000; Haider et al., 2004), and various herbal teas (Naithani and Kakkar, 2005; 2006a; 2006b). The World Health Organization has also emphasized the need to ensure quality of plant products by using modern techniques and applying suitable standards (WHO, 1998). Thus it becomes mandatory that all herbal preparations and raw materials be checked for the presence of heavy metals and residual organochlorine pesticides. Ginger, the underground rhizome of the plant *Zingiber officinale*, family *Zingiberaceae, is* the most extensively used Indian plant in traditional preparations since ancient times. It has a remarkable reputation in the treatment of many gastrointestinal disorders and is often promoted as an effective herbal antiemetic (Bone et al., 1990). It has been proposed for therapeutic uses due to its anti-inflammatory, cholesterol-lowering and anti-thrombotic properties (Ojewole, 2006). The present study deals with the estimation of selected heavy metals (Pb, Cd, Cr, Ni, As, Hg) and residual organochlorine pesticide concentrations in *Zingiber officinale* collected from various ecological zones of India with the objective to compare the variation in environmental contamination of samples collected from different locations/regions.

Materials and Methods

Rhizome of *Zingiber officinale* was collected from nine different ecological zones of India namely: Lucknow, Patna (central); Kolkata (east); Pune (west); Ottapalam, Madurai (south); Jammu, Mussoorie, and Delhi (north). Experts from the National Botanical Research Institute, Lucknow ensured the identity of all the plant samples used in this study. All chemicals used for estimation of heavy metals were of AR grade (E-Merck). The solvents used for pesticide analysis such as *n*-hexane, acetonitrile, benzene

C. Mishra · S. Sharma · P. Kakkar
Herbal Research Section, Industrial Toxicology Research Center, Mahatma Gandhi Marg, P.O. Box 80,
Lucknow 226001, India

P. Kakkar (✉)
Scientist E-II & Head, Herbal Research Section, Industrial Toxicology Research Center, Mahatma Gandhi Marg,
P.O. Box 80, Lucknow 226001, India
e-mail: poonam_kakkar@yahoo.com

were of HPLC grade. Deionized water was used throughout the study. Glassware used for the study was Borosil ‘A’ grade, including volumetric flask, conical flask, measuring cylinders, test tubes and pipettes. Plant samples were washed in fresh running water to eliminate dust, dirt and possible parasites, and then washed again with deionized water (Zurera et al., 1987). The samples were air dried prior to digestion and analysis. Analysis was carried out as per AOAC (1998) guidelines. For non-volatile heavy metals (Ni, Cd, Pb, Cr), 1 g of each powdered sample was digested with concentrated HNO_3:$HClO_4$ (6:1) in a fume chamber until white residue was obtained. The residue was dissolved and made up to 10 ml with 0.1 N HNO_3 in a volumetric flask. The solutions were then analyzed on an atomic absorption spectrophotometer (Perkin Elmer Model Analyst 300). For volatile heavy metals (Hg and As), 0.1 g of each powdered sample was digested in 10 ml concentrated H_2SO_4 overnight. The digested samples were incubated in a 70°C water bath for 1 hr. Samples were then titrated with aqueous $KMnO_4$ in an ice bath. After the solution reached room temperature, 1 ml hydroxyl ammonium chloride (20%) was added to reduce $KMnO_4$. This solution was filtered, volume made up to 100ml with milliQ water, and analyzed on an atomic absorption spectrophotometer (Varian 250 plus) with vapour generation assembly (AAS-VGA). Mixed working standard (1 and 10 μg/ml) solutions were freshly prepared by diluting the stock solutions of 1000 μg/ml (Merck India). Blanks and spiked samples were also processed and analyzed simultaneously. The detection limit of the instrument for each metal was Cr 0.0061 μg/g , Ni 0.010 μg/g, Pb 0.042 μg/g and Cd 0.0025 μg/g.

Estimation of organochlorine pesticides was carried out by taking 2 g plant material and extracting it with 150 ml of *n*-hexane using a soxhlet apparatus. The extract was passed through an anhydrous sodium sulfate filter column, which was prepared by putting about 10 g of anhydrous sodium sulfate in a glass wool column to remove traces of water. Hexane extract was then evaporated at 60°C (temperature of water bath) under reduced pressure in a rota vapor (Buchi R-114). The concentrated extract (1–2 ml) was transferred to a clean-up column with small washing of *n*-hexane. The sample was further extracted with acetonitrile/*n*-hexane saturated solvent system for oil removal followed by clean-up done with the *n*-hexane + benzene mixture elution method. Four grams of deactivated adsorbent Florisil was packed in a borosil glass column (150 × 5 mm) topped with anhydrous sodium sulphate and tapped firmly. Concentrated extract (in *n*-hexane) was transferred to the top of the column and eluted with 5 ml hexane. Elute was collected carefully and 5 ml benzene:*n*-hexane mixture (20:80) was added to the column and collected. The elute was again concentrated to 1–5 ml, as required, and analyzed on GLC (NUCON 5765) according to the standardized method in the laboratory (Naithani and Kakkar, 2006b). The temperature of the injection port (250°C), oven (190°C) and detector (250°C) was adjusted as recommended for optimum efficiency and thermal stabilization. Carrier (N_2) gas flow rate was adjusted to 60 ml/min. Five μl mixed standard solution (HCH and its isomers, α-endosulphan, DDT and its metabolite) followed by 5 μl of the samples were injected into the GLC, and chromatograms were recorded. Detection limit of equipment was 1 μg/kg, and recovery obtained under the experimental conditions was found to be 90–93%.

Results and Discussion

A total of 9 samples of *Zingiber officinale* collected from 9 different locations of India were subjected to multiresidue analysis for volatile (As and Hg), non-volatile (Pb, Cd, Ni, Cr) heavy metals and organochlorine pesticides including all the isoforms of HCH, i.e., α-HCH, *β*-HCH, *γ*-HCH, *δ*-HCH, DDT, its metabolites, and α-endosulphan. Higher concentrations of metals at a site of collection may be reflected by its concentration in plant tissues. Our data revealed that Pb was highest in the Mussoorie sample, i.e., 8.4 mg/kg, which was 8-fold more than the Ottapalam samples, which had the lowest Pb level at 0.21 mg/kg (Fig. 1a). In all other samples, Pb levels were below the permissible limit of 10 mg/kg as prescribed by

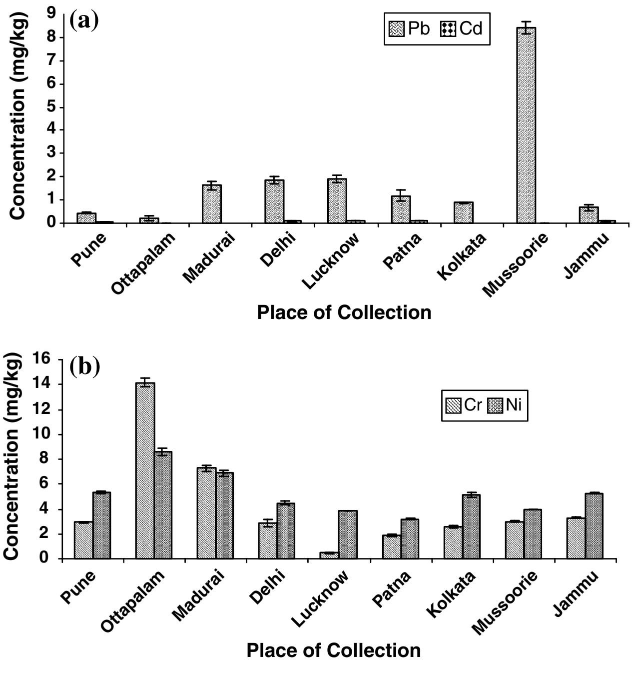

Fig. 1 **(a & b)** Non volatile metal (Pb, Cd, Cr, Ni) accumulation in *Zingiber officinale* collected from different ecological zones of India

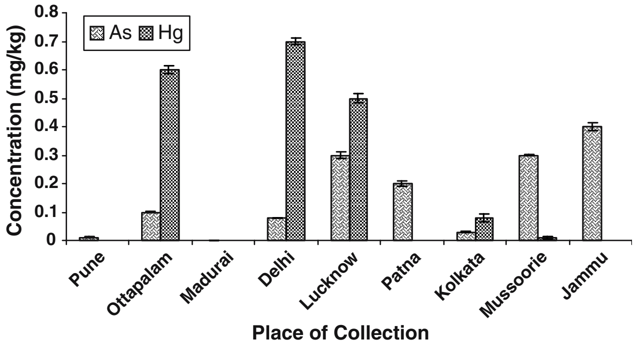

Fig. 2 Volatile metal (As, Hg) accumulation in *Zingiber officinale* collected from different ecological zones of India

Table 1 Concentrations (mg/kg) of total HCH and HCH isoformsin *Zingiber officinale* collected from different regions

Place of collection	α-HCH	γ -HCH	βHCH	δ-HCH	Total HCH
Pune	0.0789	0.2758	0.0040	BDL	0.3587
Ottapalam	0.0585	0.2034	0.0054	0.0015	0.2688
Madurai	0.0241	0.0740	BDL	0.0024	0.1005
Delhi	0.1196	0.3783	BDL	BDL	0.4979
Lucknow	0.1308	0.3910	BDL	BDL	0.5218
Patna	0.1162	0.3448	BDL	BDL	0.4610
Kolkata	0.1022	0.3392	BDL	BDL	0.4414
Mussoorie	0.2193	BDL	BDL	0.0003	0.2196
Jammu	0.0923	0.3040	BDL	BDL	0.3963

BDL, below detection limit

WHO (1998). Cd levels were highest in the Patna sample (0.09 mg/kg) and lowest in Ottapalam sample (Fig. 1a). Cd was below the detection limit in the Madurai and Kolkata samples. Even the highest level of Cd detected, i.e., in the Patna sample, was two times lower than the permissible limit. Similarly, the highest observed levels of Cr and Ni were found in the Ottapalam sample, and were 0.45 mg/kg and 8.57 mg/kg, respectively. The Madurai sample showed low levels of Cr and Ni (Fig. 1b). As and Hg (Fig. 2) were found below detection limit in the Madurai sample.

Table 1 shows the level of HCH in the samples of *Zingiber officinale* collected from different ecological zones of India. The level of α-HCH was highest in the Mussoorie sample (0.2193 mg/kg) and lowest in the Madurai sample (0.0241 mg/kg). β-HCH was detected only in the Ottapalam and Pune samples, i.e., 0.0054 mg/kg and 0.0040 mg/kg, respectively; whereas it was below detection limit in the other 7 samples of *Zingiber officinale*. The highest level of γ-HCH was detected in the Lucknow sample, which had 5 times more γ-HCH as compared to the Madurai sample, but it was below the minimal risk level (MRL) of 0.5 mg/kg and acceptable daily intake (ADI) of 0.001 mg/kg-body weight in all samples. δ-HCH was only detected in three samples: Madurai, Ottapalam and Mussoorie, with levels of 0.0024 mg/kg, 0.0015 mg/kg and 0.0003 mg/kg, respectively. The lowest concentration of total HCH was found in the Madurai sample and the highest, in the Lucknow sample at 0.5218 mg/kg. Organochlorine pesticides may find their way into the human system through food and water. Pesticides are known to enter plant products due to contamination via spillage, volatilization of residues from pesticide-treated soils, or during storage. Table 2 shows the levels of DDT and its metabolites, and the level of α-endosulphan in ginger collected from different cities. Presence of op'DDT was only found in the Delhi sample (0.0027 mg/kg), and pp' DDT was found only in the Ottapalam sample. pp' DDE, a persistent metabolite of DDT ranged from 0.0027 mg/kg (Ottapalam) to 0.0354 mg/kg (Pune) and it was found to be below detection limit in Lucknow sample. α-endosulphan was not detected in the southern region whereas all other regions showed presence of low concentrations ranging from 0.0003 mg/kg to 0.00011 mg/kg.

Table 2 Concentration (mg/kg) of α-endosulphan, DDT and its metabolites in *Zingiber officinale* collected from different regions

Place of collection	αEndosulphan	op'DDT	pp'DDT	pp'DDD	pp'DDE
Pune	0.0003	BDL	BDL	BDL	0.0354
Ottapalam	BDL	BDL	0.0214	BDL	0.0027
Madurai	BDL	BDL	BDL	BDL	0.0134
Delhi	BDL	0.0027	BDL	BDL	0.0272
Lucknow	0.0004	BDL	BDL	BDL	BDL
Patna	0.0011	BDL	BDL	BDL	0.0098
Kolkata	BDL	BDL	BDL	BDL	0.0159
Mussoorie	0.0003	BDL	BDL	BDL	0.0154
Jammu	0.0005	BDL	BDL	BDL	0.0078

BDL, below detection limit

Organochlorine pesticide residues have also been reported in human diet (Kannan et al., 1992; Dogheim, 2004), herbal preparations, spices (Srivastava et al., 2001) and in herbal teas (Naithani and Kakkar, 2004). The data clearly shows that the ginger collected from different ecological zones have low levels of heavy metal contamination and organochlorine pesticide residues. The southern region showed the lowest level of metal and pesticide contamination whereas central regions showed the highest level of contamination. Although, the pesticides like HCH and DDT are well known toxic chemicals, their residues were found at very low concentrations in all the samples of *Zingiber officinale* analyzed. This level may go even lower after processing for ayurvedic preparations as drugs or tonics. Mandatory estimations of pre-processing contaminant levels in each raw drug may help pharmaceutical companies to maintainproduct quality. The present study indicates that the *Zingiber officinale* sample from the southern region of India showed the least contamination. Generation of such data with large sample sizes may help in selection of regions for the procurement of raw drugs.

Acknowledgments The authors are thankful to Dr. C.M. Gupta, Director of the Industrial Toxicology Research Center, Lucknow for his interest in this work. Support extended by the Analytical section of ITRC in carrying out estimations is also gratefully acknowledged.

References

AOAC (1998) Wet digestion for non-volatile metals. In AOAC official methods of analysis, vol 1, 16th edn. AOAC International, Gaithersburg, MD, ch9

Bone ME, Wilkinson DS, Young JR (1990) Ginger root: new antiemetic, the effect of ginger root on prostoperative nausea and vomiting after major gynaecological surgery. Anaesthesia 45:669–671

Dogheim SM (2004) Pesticides and heavy metals levels in Egyptian leafy vegetables and some aromatic plants. Food Add Cont 21:323–330

Haider S, Naithani V, Barthwal J, Kakkar P (2004) Heavy metal content in some therapeutically important medicinal plants. Bull Environ Contam Toxicol 72:119–127

Kannan K,Tanabe S, Quynh HT, Hue ND, Tatsukhawa R (1992) Residue pattern and dietary intake of persistent organochlorine compounds in foodstuffs fromVietnam. Arch Environ Contam Toxicol 22:367–374

Ojewole JA (2006) Analgesic, antiinflammatory and hypoglycaemic effects of ethanol extract of *Zingiber officinale* Rosc. rhizomes (Zingiberaceae) in mice and rats. Phytother Res 20:764–772

Rai V, Kakkar P, Khatoon S, Rawat AKS, Mehrotra S (2001) Heavy metal accumulation in some herbal drugs. Pharmaceut Biol 39:384–387

Srivastava LP, Budhwar R, Raizada RB (2001) Organochlorine pesticide in Indian spices. Bull Enviorn Contam Toxicol 67:856–862

Naithani V, Kakkar P (2004) An evaluation of residual organochlorine pesticides in popular herbal teas. Arch Environ Health 59:426–430

Naithani V, Kakkar P (2006a) Effect of ecological variation on heavy metal content of some medicinal plants used in herbal tea ingredients in India. Bull Environ Contam Toxicol 76:285–292

Naithani V, Kakkar P (2006b) Estimation of organochlorine pesticide residue in two popular spices extensively used as herbal tea ingredients in India. Bull Environ Contam Toxicol 76:429–435

Patwardhan B, Warude D, Pushpangadan P, Bhatt N (2005) Ayurveda and traditional Chinese medicine: a comparative overview. Evid Based Complement Alternat Med 2:465–473

World Health Organization (1998) Quality control methods for medicinal plant material. WHO, Geneva, Switzerland

Zurera G, Estrada B, Rincon F, Pozo R (1987) Lead and cadmium contamination levels in edible vegetables. Bull Environ Contam Toxicol 38:805–812